

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Lovell-Badge et al.

Serial No.: Not Yet Assigned

Filed: June 21, 2001

Entitled: Cell Lineage Markers

Examiner: Not Yet Assigned

Art Unit: Not Yet Assigned

Atty. Docket No.: 18396/2032

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please enter amended claims 1-17 as follows.

Claims

1. A method for isolating a pluripotent cell which is at least partially committed to a given developmental pathway comprising the steps of:
 - (a) selecting a population of pluripotent cells;
 - (b) detecting *Sox* gene expression;
 - (c) sorting the cells according to *Sox* gene expression; and
 - (d) isolating those cells which express a *Sox* gene.
2. The method of claim 1, wherein said population of cells is derived from CNS tissue.
3. The method of claim 1, wherein said population of cells is derived from a cell culture.

4. The method of claim 1, wherein said *Sox* gene expression is detected by nucleic acid hybridization.
5. The method of claim 1, wherein said *Sox* gene expression is detected by binding of a SOX polypeptide or a SOX nucleic acid corresponding to mRNA to a detectable ligand.
6. The method of claim 5, wherein the detectable ligand is a labeled immunoglobulin.
7. The method of claim 5, wherein said detectable ligand is a labeled oligonucleotide complementary to *Sox* mRNA.
8. The method of claim 1, wherein said *Sox* gene expression is detected by FACS analysis.
9. A method for isolating a desired cell type from a population of cells, comprising the steps of:
 - (a) transfecting said population of cells with a genetic construct comprising a coding sequence encoding a detectable marker operatively linked to control regions sensitive to modulation by a SOX polypeptide;
 - (b) detecting the cells which express said detectable marker; and
 - (c) sorting said cells which express said detectable marker from said population of cells.
10. A method for isolating a neuroblastic cell from a population of cells, comprising the steps of:
 - (a) transfecting said population of cells with a genetic construct comprising a coding sequence encoding a detectable marker operatively linked to a control sequence which is transactivatable by a SOX polypeptide;
 - (b) detecting the cells which express said detectable marker; and
 - (c) sorting said cells which express said detectable marker from said population of cells.

11. The method of claim 9 or claim 10, wherein said detectable marker is a fluorescent or luminescent polypeptide.

12. The method of claim 9 or claim 10, wherein said detectable marker is a polypeptide detectable at the surface of the cell.

13. A method for producing a cell committed to a specified lineage, comprising the steps of:

- (a) transfecting a pluripotent stem cell with a genetic construct comprising a coding sequence expressing a SOX polypeptide;
- (b) culturing said stem cells to differentiate them into neural cells; and
- (c) isolating said neural cells thereby produced.

14. The method of claim 13, wherein said coding sequence expressing a *Sox* polypeptide is operatively linked to an inducible promoter.

15. The method of claim 13 or 14, wherein said cell is further transfected with a vector comprising a sequence encoding a regulator which regulates the expression of the *Sox* sequence.

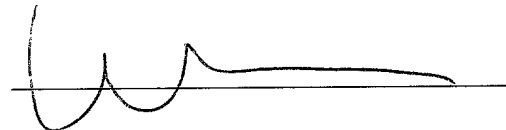
16. The method of claim 1, 9 or 13, wherein said *Sox* gene is a member of *Sox* Group A.

17. The method of claim 16, wherein said *Sox* gene is *Sox1* or *Sox2*.

Respectfully submitted,

6/21/01

Date



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MARKED UP AMENDED CLAIMS

Sir:

Please amend the following claims as follows.

1. A method for isolating a pluripotent cell which is at least partially committed to a given developmental pathway, comprising the steps of:

(a) selecting a population of pluripotent cells;

(b) detecting Sox gene expression

[(b)] (c) sorting the cells according to Sox gene expression; and

[(c)] (d) isolating those cells which express a [given] Sox gene.

2. The [A] method [according to] of claim 1, wherein [the] said population of cells [for] is derived from CNS tissue.

3. The [A] method [according to] of claim 1, wherein [the] said population of cells is derived from a cell culture.

4. The [A] method [according to any preceding] of claim 1, wherein [the] said [expression of the] *Sox* gene expression is detected by nucleic acid hybridization.

5. The [A] method [according to any one] of claim[s] 1 [up to 3], wherein [the] said [expression of the] *Sox* gene expression is detected by [a] binding of a SOX polypeptide or a SOX nucleic acid corresponding to mRNA to a detectable ligand.

6. [A] The method [according to] of claim 5, wherein [the] said detectable ligand is a labeled immunoglobulin.

7. [A] The method [according to] of claim 5, wherein [the] said detectable ligand is a labeled oligonucleotide complementary to *Sox* mRNA.

8. [A] The method [according to any preceding] of claim 1, wherein [the expression of the] said *Sox* gene expression is detected by FACS analysis.

9. A method for isolating a desired cell type from a population of cells, comprising the steps of:

- (a) transfecting [the] said population of cells with a genetic construct comprising a coding sequence encoding a detectable marker operatively linked to control regions sensitive to modulation by a SOX polypeptide;
- (b) detecting the cells which express [the] said [selectable] detectable marker; and
- (c) sorting [the] said cells which express [the] said [selectable] detectable marker from [the] said population of cells.

10. A method for isolating a neuroblastic cell from a population of cells, comprising the steps of:

- (a) transfecting [the] said population of cells with a genetic construct comprising a coding sequence encoding a detectable marker operatively linked to a control sequence which is transactivatable by a SOX polypeptide;
- (b) detecting the cells which express [the] said [selectable] detectable marker; and

- (c) sorting [the] said cells which express [the] said [selectable] detectable marker from [the] said population of cells.

11. [A] The method [according to] of claim 9 or claim 10, wherein [the] said [selectable] detectable marker is a fluorescent or luminescent polypeptide.

12. [A] The method [according to] of claim 9 or claim 10, wherein [the] said [selectable] detectable marker is a polypeptide detectable at the surface of the cell.

13. A method for producing a cell committed to a specified lineage, comprising the steps of:

- (a) transfecting a pluripotent stem cell with a genetic construct comprising a coding sequence expressing a SOX polypeptide;
- (b) culturing [the] said stem cells in order to differentiate them into neural cells; and
- (c) isolating [the] said neural cells thereby produced.

14. [A] The method [according to] of claim 1[5]3, wherein [the] said [*Sox*] coding sequence expressing a Sox polypeptide is operatively linked to an inducible promoter.

15. [A] The method [according to] of claim 13 or claim 14, wherein [the] said cell is further transfected with a vector comprising a sequence encoding a regulator which [modulates] regulates the expression of the *Sox* sequence.

16. [A] The method [according to any preceding] of claim 1, 9 or 13, wherein [the] said *Sox* gene is a member of *Sox* Group A.

17. [A] The method [according to] of claim 16, wherein [the] said *Sox* gene is *Sox1* or *Sox2*.